

DESTRUCTION OF PHYTATE IN A WET MIXTURE
OF SOYBEAN MEAL, GROUND CORN AND BRAN

by

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INTRODUCTION AND LITERATURE REVIEW

Phytic acid is myo-inositol (a cyclohexitol) esterified on each alcoholic group with orthophosphoric acid. Phytate, which is the term used for salts of phytic acid, is the major storage form of phosphorus in plants (Maga, 1982; Cheryan, 1980; Cosgrove, 1980; Huang, 1983; Nelson et al., 1968a).

Nelson et al. (1968a) investigated the levels of phytic acid in numerous feed ingredients. They reported 0.6%, 0.7%, and 3.4% phytic acid respectively in corn, milo, and wheat bran. Lolas and Markakis (1975) found 1 to 1.47% phytic acid in 15 varieties of whole soybeans, while 0.72% to 1.8% was reported in defatted meal from over 20 varieties of soybeans (Anon., 1976). In 18 varieties of barley, phytic acid ranged from 0.97 to 1.08%, and in 19 varieties of oats, 0.84 to 1.01% (Lolas et al., 1976). Defatted glandless cottonseed, which is also a feed ingredient, was found to contain 2.9% phytic acid (Wozenski and Woodburn, 1975). Harland and Prosky (1979) reported 1.46% phytic acid in rye. The levels of phytate in other commodities have been given by Oberleas (1983).

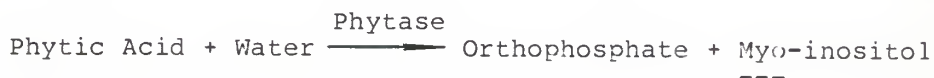
Phytic acid strongly binds essential dietary minerals, such as calcium, zinc, magnesium, iron, and copper, making them unavailable or partially unavailable for absorption (Maga, 1980; Cosgrove, 1980; Atwal et al., 1980; O'Dell et al. 1972; Cheryan, 1980; Erdman, 1979). Phytate may decrease bioavailability of divalent and trivalent minerals by interacting directly with the cations. Alternatively, phytate forms multicomponent complexes with proteins and metal ions. Those multicomponent complexes may inhibit mineral absorption under physiological conditions

(Cheryan, 1980).

Phytate is thought to bind to protein to form soluble or insoluble complexes (Cheryan, 1980; Hill & Tyler, 1954b; Prattley et al., 1982b; Omosaiye and Cheryan, 1979). At low pH, most proteins possess a net positive charge, while phytic acid has two or more negatively charged phosphate groups above pH 1.5. Many proteins and phytic acid interact at low pH due to strong electrostatic forces. At alkaline pH's, most proteins carry a net negative charge. Cations appear to form an electrostatic bridge between negatively charged protein and phytate. In the absence of protein at high pH, many divalent metal salts of phytate are insoluble, such as the calcium and magnesium salts (Cheryan, 1980; Saio et al., 1968; McKinney et al., 1949; Smith and Rackis, 1957).

A protein-phytic acid complex could explain the reported trypsin inhibitor activity of phytate (Singh and Krikorian, 1982).

Phytic acid can be hydrolyzed by a phosphatase enzyme called phytase. This enzyme hydrolyses phosphate linkages of phytic acid to release orthophosphate and myo-inositol. The reaction is as follows:



Phytase was first found in rice in 1907 by Suzuki et al. (1907). Recently, phytase activity in rice was shown to be concentrated in globoid bodies in the aleurone cells (Tanaka and Kasai, 1981). Hart et al. (1909) found that soaking wheat bran

overnight in water allowed the enzyme phytase to act, thereby releasing myo-inositol and inorganic phosphorus. McCance and Widdowson (1944) and Mollgaard (1946) demonstrated that wheat, rye and barley were high in phytase activity. Nagai and Funahashi (1962) reported that the phytase in wheat bran was a nonspecific acid-phosphomonoesterase. Greaves et al. (1967) indicated that an enzyme specific for hydrolysing myo-inositol triphosphates is uncertain since phytase was active on glycerophosphate. Their comments suggest that phytase in bran is selective for phytate but not specific.

In growing chickens, Nelson et al. (1968b) reported that when no phytate was present, a calcium level of 0.5% was needed for optimum growth. However, when 1.25% phytate was present in a natural diet, the calcium requirement in the feed increased to 0.95%.

Abrams et al. (1975) and Cornelius and Harmon (1974) found that pigs fed unsupplemented, ensiled, high moisture corn gained significantly faster and more efficiently than those on unsupplemented dry corn. Apparently, ensiling corn gave some phytate destruction.

Phytase activity has been found in the mucosa of the small intestine of the pig, but it is generally agreed that utilization of phytate by pigs is limited (Huang, 1983). From the results of experiments comparing two supplemental sources of calcium, dicalcium phosphate (DCP) and calcium phytate (CaPp), Pierce et al. (1976) reported that grower pigs fed DCP in the growing period gained faster ($p < 0.05$) than those fed CaPp and had a significantly better feed to gain ratio ($p < 0.01$). These results

suggest that CaPp as a supplemental source of phosphorus adversely affected the performance and bone development in pigs. It appears important to find a method to produce low-phytate feed for monogastric animals.

The first objective of this study was to use naturally occurring phytase in wheat bran to simultaneously hydrolyze phytate in a mixture of soybean meal and bran. The second objective was to use the phytase in wheat bran and corn to destroy phytate in a mixture of soybean meal, ground corn, and wheat bran. The third objective was to study phytate loss in ensiled whole corn.

EXPERIMENTAL PROCEDURE

Raw Materials and Reagents

Anhydrous sodium carbonate (Mallinckrodt, Inc., St. Louis, MO), concentrated hydrochloric acid (Fisher Scientific Company, Fair Lawn, NJ), sodium hydroxide (Taylor Chem. Company, St. Louis, MO), ethylenediamine-tetraacetic acid (EDTA, 99% pure, Sigma Chem. Company, St. Louis, MO), oxalic acid dihydrate (97% pure, Aldrich Chem. Co., Inc., Milwaukee, WIS), and 85% lactic acid (MCB Manufacturing Chemists, Inc., Cincinnati, Ohio) were all reagent grade chemicals.

Defatted soybean meal (SBM) came from the Farmers Co-op Assn, Manhattan, KS. The SBM had been produced by de-fatting soybean flakes by solvent extracting, de-solventizing, toasting (above 100 °C, see Brueske, 1976), and grinding the flakes with a hammermill. The average particle size of the ground SBM was 1.03

mm with a distribution of 16% over a 10-mesh (1.7mm) sieve, 32% over a 14-mesh (1.2mm) sieve, 21% over a 20-mesh (0.8mm) sieve, 14% over a 28-mesh (0.6mm) sieve, and 16% through a 35-mesh (0.4mm) sieve, as determined by the method of Pfoest and Headley (1976). The composition of SBM is given in Table I. It was stored at 4 C prior to use.

Ground yellow-dent corn and whole corn were obtained from the Pilot Feed Mill at the Department of Grain Science, KSU. The average particle size of ground corn was 0.69mm with a distribution of 5% over a 10-mesh (1.7mm) sieve, 20% over a 14-mesh (1.2mm) sieve, 20% over a 20-mesh (0.8mm) sieve, 16% over a 28-mesh (0.6mm) sieve, 14% over a 35-mesh (0.4mm) sieve, 6% over a 48-mesh (0.3mm) sieve, 17% over a 70-mesh (0.2mm) sieve, and 1% through a 100-mesh (0.1mm) sieve. The composition of the ground corn is given in Table I. Ground corn was stored at 4 C prior to use.

Wheat bran (bran) was obtained from the Pilot Flour Mill at the Department of Grain Science, KSU. The average particle size of the bran was 1.69mm with a distribution of 15% over a 8-mesh (2.4mm) sieve, 37% over a 10-mesh (1.7mm) sieve, 35% over a 14-mesh (1.2mm) sieve, and 12% through a 20-mesh (0.8mm) sieve. The composition of bran is given in Table I. It was stored at 4 C prior to use.

Inorganic Phosphorus (Pi) Determination

The Pi in the sample was determined (Pons et al., 1946) by extracting the sample (5g) with 100ml of 12.3% aqueous trichloroacetic acid on a mechanical shaker for 12h at room temperature. After centrifugation at 2,000Xg using a Beckman

Model J-21 centrifuge for 30min, an aliquot (5.0ml) was diluted to 100ml with distilled water. The solution was then analyzed directly for orthophosphate by the method of Lindberg and Ernster (1956) as modified by Nahapetian and Bassiri (1975). A standard curve was prepared having concentrations of 1.98×10^{-5} to 4.96×10^{-5} mg of phosphorus per 2.0ml using reagent-grade potassium dihydrogen phosphate.

Phytate Phosphorus (Pp) Determination

The procedure for determining phytate phosphorus (Pp) was that described by Tangkongchitr et al. (1980a). Five grams of a sample were extracted with 100ml of 1.2% aqueous HCl containing 10% Na_2SO_4 . The mixture was shaken at room temperature for 24h and centrifuged for 40 min at 2,000Xg using a Beckman Model J-21 centrifuge. An aliquot (10ml) of the clear supernatant was taken, and the phytic acid in the extract was precipitated by adding 5ml of aqueous 0.4% ferric chloride hexahydrate in 0.6% HCl containing 5% Na_2SO_4 . The mixture was heated in a boiling water bath for about 20 min to complete precipitation. The ferric phytate precipitate was collected as a solid pellet by centrifuging at 2,600Xg for 30 min using the Beckman centrifuge. The supernatant was discarded.

The yellowish pellet was dissolved in a mixture of concentrated H_2SO_4 (2ml) and concentrated HNO_3 (3ml), and transferred quantitatively to a micro-Kjeldahl flask for wet combustion. The digestion, which usually required about 20 min, was judged complete when white fumes filled the flask and hung over the liquid. Distilled water (10ml) was slowly added to the

acid digest while it was still warm, and the aqueous solution was boiled in a water bath for 30 min. The solution was then transferred quantitatively with distilled water, and the volume was brought to 100ml. The phytate phosphorus was determined colorimetrically as previously described.

Destruction of Phytate in Bran, SBM and Ground Corn Alone

Throughout the work reported here, when the destruction of phytate in the ingredients was examined, either a binary ingredient mixture of 24g of SBM and 6g of bran (total 30g) or a ternary ingredient mixture of 12g corn, 12g SBM and 3g bran (total 27g) was used. The weights of the ingredients used in the experiments was on an "as is" moisture basis. It should also be mentioned that the pH's reported for mixtures are the final equilibrium pH's reached. Oftentimes during pH adjustment, the initial pH of a mixture was higher or lower than the equilibrium pH. Equilibrium was reached about 30 min after adjustment.

Bran (triplicate samples, 30g each) was mixed with water (96.5ml) and 3.5ml of lactic acid (17.2% aqueous solution), which caused the pH in the wet bran to decrease from an initial value of 6.8 to pH 5.0 after 30 min. The mixture was placed in a water bath at 45 C for 6h, after which time the pH of the reaction medium remained at about pH 5. The sample was dried at 80 C in an oven for about 3.5h, and phytate phosphorus and moisture were determined.

The same procedure was used to treat either SBM (30g) or ground corn (30g) alone, except that 95.2ml of distilled water plus 4.8ml of 17% lactic acid were used for SBM and 97ml of distilled water plus 3ml of HCl (5N) were used for corn. A

single sample of bran, corn and SBM was examined for Pp destruction.

Destruction of Phytate in a Binary Mixture of SBM and Bran or a

Binary Mixture of Ground Corn and Bran

Bran (6g) and SBM (24g) were added to 95.5ml of distilled water and 4.5ml of 17% lactic acid, and after about 30min the mixture gave pH 5. Ground corn (24g) and bran (6g) were mixed with 97ml of distilled water plus 3ml of HCl (5N), and after 30min, this mixture also gave pH 5. Both mixtures were incubated at 45 C for 6h. After the enzymolysis reaction, the pH's of both reaction mixtures were about 5.1. The destruction of phytate was determined.

Destruction of Phytate in a Binary Mixture of Bran and

Conditioned SBM

Phytate in SBM was destroyed using a two-step procedure. In step I or the conditioning step, three experiments were conducted to find the conditions that "released" the most phytate from SBM. The "released" phytate was then enzymically hydrolyzed in step II using bran as the source of phytase.

In experiment 1, SBM (24g) was mixed with water (70ml), then 5N HCl or 2N NaOH was added to obtain pH 2-10 after 30min (Appendix). The final volume of the aqueous mixture was adjusted to 100ml with water. Conditioning was done at different temperatures (45 C, 55 C, 65 C) and for different time periods (2h, 3h, 4h).

In experiment 2, the conditioning procedure was the same as that in experiment 1 except Na_2CO_3 was used in place of NaOH to

adjust pH to 7.5-9.1.

In experiment 3, SBM (24g) was mixed with water (70ml), and calcium chloride (1.68g) was added together with 5N HCl to obtain pH 4 after 30min. A second identical sample was prepared at pH 5. The final volume of both wet mixtures was adjusted to 100ml by adding water, and both mixtures were placed in a water bath at 45 °C for 2h.

In step II or the enzymolysis step, all the samples from the conditioning experiments 1, 2 and 3 were treated identically. A conditioned SBM mixture was adjusted to pH 5 by adding 5N HCl or 2N NaOH, which amounted to between 0.75ml and 32ml. Bran (6g) was added to the aqueous slurry containing the conditioned SBM, and the mixture thoroughly blended by stirring with a glass rod. Thus, the ratio of solid ingredients to water in the enzymolysis reaction ranged from 1:2 to 1:3 for the various samples. The pH value was checked 30 min after beginning the enzymolysis reaction. In all samples, the pH was 5.0 ± 0.2 . Each mixture was placed in a water bath at 45 °C for 6h. After enzymolysis, the sample was dried at 80 °C in a forced-draft oven for about 3.5h, and then assayed for moisture and Pp. During the enzymolysis reaction on some samples, EDTA (0.29g) or oxalic acid (0.18g) was added, in which case the enzymolysis period was increased from 6h to 8h.

Destruction of Phytate in a Ternary Ingredient Mixture Containing Bran, Ground Corn and SBM; Preconditioning of SBM Alone before Enzymolysis

Into each of seven beakers was placed SBM (12g), oxalic acid (0.09g or 0.18g) and water (100ml), and 5N HCl or solid Na_2CO_3

was added to obtain pH 4.5, 5.5, 6.5 or 8.5. Then, the beakers were placed in a water bath at 55 C for 2h, and after conditioning, the mixtures were brought to pH 5 using HCl (5N) or NaOH (2N). Ground corn (12g) and bran (3g) were added, the mixtures stirred by hand using a glass rod, and incubated at 45 C for 8h. The ratio of solid-ingredients to water in the enzymolysis reactions was 1:3.3. After drying at 80 C in an oven, the samples were assayed for moisture and Pp.

Destruction of Phytate in a Ternary Ingredient Mixture of Bran, Ground Corn and SBM; Conditioning of SBM and Corn in Separate Containers

SBM (12g) and oxalic acid (0.09g) were combined with 50ml water, and the mixture adjusted to pH 4.5 by adding 0.2ml 5N HCl. Nine identical samples were prepared, and all were conditioned at 55 C for 2h. Into nine other beakers, ground corn (12g) and oxalic acid (0.09g) were added to 50ml water. Six of the corn samples were adjusted pH to 3 (three samples) and 3.5 (three samples) using 5N HCl, while the other three were adjusted to pH 4 using Na_2CO_3 . The corn samples were then conditioned for 1h, 2h, or 3h at 55 C.

After conditioning, the corn and SBM slurries were combined, and the pH of the mixture adjusted to pH 5 by adding 2N NaOH. Bran (3g) was added, which did not affect pH, and the mixture was incubated 8h at 45 C. The rest of the procedure was the same as before.

Destruction of Phytate in a Ternary Ingredient Mixture Containing Bran, Ground Corn and SBM; Conditioning of a Mixture of Corn and

SBM or Sequential Conditioning of Corn and SBM Together

A mixture containing SBM (12g) and ground corn (12g) was added to water (100ml) containing 0.18g oxalic acid. The slurry was adjusted to pH 4.5, 5.5, 6.5 by adding 5N HCl or Na_2CO_3 and the mixture incubated at 55 °C for 2h. All the mixtures were adjusted to pH 5 using 5N HCl or 2N NaOH, and then bran (3g) was added with stirring. After an incubation period of 8h at 45 °C, the samples were dried at 80 °C, and assayed for moisture and Pp. The ratio of solid-ingredients to water was 1:3.3 in the enzymolysis step.

Alternatively, corn and SBM were sequentially conditioned in one container. Ground corn (12g) and oxalic acid (0.18g) were mixed with 100ml of water to give a slurry with pH about 3. Two other samples of ground corn (12g each) were separately mixed with oxalic acid (0.18g) and 100ml of water, adjusted to pH 3.5 and 4 using Na_2CO_3 , respectively. Each mixture was stored at 55 °C for 2h, while three other identical samples were prepared and stored at 65 °C for 2h. SBM (12g) was then added to all six samples, which increased pH above 5.5. The pH was readjusted to 4.5 by adding 5N HCl, and the six mixtures containing SBM and corn were conditioned for another 2h at 55 °C.

After the conditioning step, 5N NaOH was added to adjust pH to 5, bran (3g) added, and the ternary ingredient mixture was incubated 8h at 45 °C. The ratio of solid-ingredients to water in the enzymolysis reaction was 1:3.3. After the enzymolysis reaction, the mixture was dried and assayed for moisture and Pp.

Destruction of Phytate during Ensiling of Whole Corn

Four samples of whole yellow-dent corn (4.6kg each, 14.3%

moisture) were placed each in a double plastic bag, and about 340ml of water was poured into each bag. After 6-8h standing at 20 °C, two more aliquots (2X340ml) of water were added in the same manner, and the moisture in the corn increased to about 30% (w.b.). The bags were tied shut and placed in a hardboard container, and the container stored in a room held at 20 °C. After 1, 2, 3 and 5 weeks storage, a bag was removed from storage, and the corn at the top, center and bottom of a bag was analyzed for moisture, Pp and Pi.

RESULTS AND DISCUSSION

Destruction of Phytate in Bran, SBM and Ground Corn When Each Ingredient Was Wet Alone to pH 5, or When Mixtures of Bran and SBM or Bran and Corn Were Wet to pH 5

A formula feed for growing chicks often contains soybean meal (SBM), ground corn, and bran, all of which contain phytic acid as shown in Table I. Assuming the weight proportion of these ingredients in the feed are 4:4:1 for SBM:corn:bran (Allee, 1984), the contribution of each ingredient to the total phytate in the feed is 47%, 23% and 30% for SBM, corn and bran, respectively (Table I).

When bran (1 part) was wet with 0.12 parts of 0.6% (based on water) aqueous lactic acid, the pH of the mixture equilibrated to about pH 5 in 30 min. After incubation of the wet bran for 6h at 45 °C, 91% of the phytate phosphorus (Pp) was lost (Table II). Thus, the phytate in bran was accessible to the indigenous phytase or phosphatase enzyme(s). On the other hand, incubation

of wet SBM or ground corn alone at pH 5 and 45^o for 6h gave only 10-23% loss of Pp, indicating SBM and corn contained a low level of phytase or else the phytate was inaccessible to phytase.

When ground corn was incubated together with bran at pH 5, 51% of Pp in the mixture was destroyed, which indicated ground corn contained a low phytase activity. When SBM was incubated with bran, phytate destruction was 37%, indicating the phytate in SBM was not as accessible to phytase from bran.

Wu et al. (1984) previously reported that when bran was mixed with water and acid at a bran:water ratio of about 1:2, phytate destruction was optimum at pH 5.1 and 45^o C. After 6h at optimum pH and temperature, Wu et al. (1984) found 93% destruction of phytate in bran. Furthermore, those workers reported more complete loss of Pp as the water to bran ratio was increased from 2:1 to 3.3:1. The optimum for phytase activity at pH 5 agrees well with that found by Tangkongchitr et al. (1980b), Nagai and Funahashi (1962) and Hill & Tyler (1954a). Other workers have reported similar values for the optimum pH of phytase activity, including pH 5.15 (Peers, 1953), 5.2 (Gibbins and Norris, 1963), and 5.3 (Lolas and Markakis, 1977).

The optimum temperature of 45^o C for phytase hydrolysis found in this study and by Wu et al. (1984) agreed with that reported by Nagai and Funahashi (1962), although it is a little below the 50^o C given by Hill & Tyler (1954a). Peers (1953) reported 55^o C as the optimum temperature for phytase activity.

When the pH in the enzymolysis reaction was adjusted using lactic acid, and the enzymolysis reaction time at 45^o C extended beyond 6h, mold growth was seen on the bran. If hydrochloric acid

was used in place of lactic acid to adjust pH to 5, no mold was visible after 8-12h of enzymolysis. It seems that the strongly acidic HCl killed fungal spores before the acid was buffered by the bran protein.

As already stated, when wet SBM (SBM:water=1:3.2) was incubated alone at pH 5 and 45^o C for 6h, little loss (10%) of phytate occurred (Table II). Apparently, practically all the phytase enzyme was denatured when the defatted soybean flakes were heat-treated to destroy trypsin inhibitor. It would be interesting to determine if defatted soy flakes could be heat-treated under mild conditions where trypsin inhibitor might be denatured but the enzyme phytase preserved. The optimum temperature for phytase activity of about 45-50^o C indicates that it might be possible to preserve phytase and selectively destroy trypsin inhibitor.

Conditioning SBM Followed by Enzymolysis of a Mixture of Conditioned SBM and Bran

It appears that the phytate in SBM is not readily accessible to the phytase in bran. Thus, the SBM was conditioned to release phytate. The term "conditioning" or "preconditioning" was used to denote the wetting of ingredients (SBM, and ground corn) with aqueous solutions of reagents and holding the wet ingredients at different pH's, temperatures, and time periods. It was assumed that little loss of Pp occurred during the conditioning step, since conditioning was done at extremes of pH, and since the phytase activity was low in SBM and in corn.

A number of investigators have pointed out that there is

often strong binding between phytic acid and protein (Okubo et al., 1976; Prattley and Stanley, 1982a; Cheryan, 1980; Maga, 1982; de Rham and Jost, 1979; Hill & Tyler, 1954b). Okubo et al.(1976) indicated that the binding is electrostatic and involves the anionic phosphate groups of phytate and the cationic groups of proteins. Prattley and Stanley (1982a) studied the location of phytate in soybeans. They found that phytic acid was located in protein bodies distributed uniformly throughout the cotyledons. The phytate occurred in the form of a soluble protein-phytate complex inside globoid inclusions. Hence, phytate is deposited in soybeans in close association with protein. It seems likely that when soybeans are defatted, the subsequent heat-treatment destroys phytase activity in the SBM, since the water-solubility of most of the protein in the meal is lost (Belter and Smith, 1952, Kellor, 1974). Thus, it appears that the phytate in SBM remains in close association with water-insoluble proteins in SBM.

In order to destroy phytate in SBM, a two step procedure was investigated. In step I, SBM was conditioned to release phytate, and in step II, the conditioned SBM (4 parts) was adjusted to pH 5, and mixed with 1 part of bran. The mixture was then incubated at 45 C for 6h to hydrolyze the phytate. The loss of Pp was calculated as a percentage of the total Pp in the mixture. Total Pp in the mixture was 0.7% on a dry solids basis, since 4 parts of SBM containing 0.54% Pp was mixed with 1 part of bran containing 1.4% Pp.

Table III gives the loss of phytate phosphorus when SBM was conditioned between pH 2 and 10 using HCl or NaOH to adjust pH.

From the data in Table III, a pH of 4 or 8 was found to give the highest release (56-58%) of phytate from SBM. Ford et al.(1978) reported an optimum pH of 5.0 to 5.5 to prepare a low-phytate lipid-protein concentrate from full-fat soy flour, while de Rham and Jost (1979) used pH 5.5 with no sodium chloride to dephytinize defatted, enzyme-active soy flour. Apparently, the optimum pH to release phytate from the denatured protein in SBM is different from that required for native soy proteins.

The soaking temperature to release phytate from wet SBM was not as important as pH. At pH 8, increasing the temperature from 45 C to 65 C increased phytate loss by only 4 percentage points (Table III). Maga (1982) stated that complexes of protein with phytate are heat-labile above pH 8. However, Prattley et al.(1982b) indicated that heating a soy protein-phytate complex caused no apparent change in the amount of bound phytate even at 100 C. Our results agree with Prattley's. Consequently, 55 C was decided as the conditioning temperature for SBM.

It was also observed that extending the time period of conditioning wet SBM beyond 2h gave little or no increase in phytate destruction (Table III). For example, at 45 C and pH 4, soaking for 2h and 3h gave 58% and 57% loss of phytate, respectively. Therefore, 2h was considered sufficient for conditioning SBM.

In order to increase phytate destruction above 60%, several other factors were investigated in the conditioning step. Since Ca^{++} and Mg^{++} ions are known to insolubilize phytate or to bind phytate to protein, sodium carbonate was added to SBM during

those assumptions, the amounts of EDTA and oxalic acid were calculated to be 1.2 parts and 0.75 parts, respectively, for 100 parts of SBM, and 0.97 parts and 0.6 parts for 100 parts of a 4:1 mixture of SBM and bran.

When SBM was conditioned 2h at pH 8.5 and 55 °C with a water:Na₂CO₃:SBM ratio of 4:0.2:1, the conditioned SBM mixed with bran containing EDTA, and the mixture then adjusted to pH 5 and incubated 6h at 45 °C, 86% of phytate phosphorus was lost. When oxalate was added with bran under the same conditions, 74% of phytate phosphorus was lost (Table IV). Thus EDTA and oxalate increased the destruction of Pp above the 71% observed for the control.

The use of EDTA in the chick feed does not appear advisable because of its ability to chelate minerals in the digestive tract, thereby, defeating the objective of destroying phytate. The use of oxalic acid in a chick feed may be tolerable. In humans, Liener (1980) stated that high levels of oxalic acid may interfere with calcium metabolism. More information is needed to evaluate the use of oxalic acid in chick feed.

Conditioning SBM with Added Calcium Ion

Okubo et al.(1976) observed a greatly improved rate of removal of phytate from hexane-defatted soybean meal by dialysis and diafiltration at pH 3 in the presence of 0.5 M CaCl₂. Ford et al.(1978) recommended pH 3.5 to pH 4.0 and 0.04 M CaCl₂ to obtain a 90% removal of phytic acid from whole raw soybeans.

In this work, the amount of Ca⁺⁺ added to SBM during conditioning was calculated according to the requirement of Ca

for growing chickens (The National Research Council, 1977). The amount calculated was 7g of CaCl_2 for 100g of SBM. Using a SBM:water: CaCl_2 ratio of 14:53:1 (w:w:w), SBM was conditioned either at pH 4 and 45 C, or pH 5 and 45 C. After conditioning the SBM with the CaCl_2 solution, the conditioned SBM (24g) was then mixed with bran (6g), and the native phytase allowed to act for 6 h at 45 C and pH 5. The phytate losses were 18% and 36% of the total Pp in the mixture of SBM and bran when the SBM- CaCl_2 mixture was conditioned at pH 4 and pH 5, respectively. The losses in the control samples at pH 4 and pH 5 without Ca^{++} were 58% and 49%, respectively. It appears that in raw soybeans calcium causes release of a soluble phytate protein complex during conditioning at pH 3-4, but in SBM Ca^{++} probably causes the phytate to bind to the insoluble protein. The insoluble calcium-phytate-protein in SBM is unaffected by phytase in the enzymolysis step.

Conditioning SBM with Oxalate at pH 4.5-8.5 and Enzymolysis of

 the Ternary Ingredient Mixture Containing Conditioned SBM, Ground

 Corn and Bran

In a feed containing a 4:4:1 mixture of SBM, corn and bran, corn contributes approximately one-fourth of the phytate in the mixture (Table I). Therefore, work was done to destroy phytate in the ternary mixture of ingredients.

In work done on the binary mixture of SBM and bran (Table IV), it was observed that phytate loss was higher (74% vs 69%) when oxalic acid was added at the enzymolysis step at pH 5 than when oxalic acid was added at the conditioning step at pH 8.5.

The same was true for EDTA (86% vs 73%, Table IV). For that reason, it was decided to investigate the effect of pH 4.5, 5.5, 6.5, and 8.5 on the conditioning of SBM in the presence of oxalic acid.

Mixtures of SBM:oxalic acid:water at 133:1:1111 and 67:1:556 were conditioned at 55 C for 2h at pH's between 4.5 and 8.5. After adjusting the pH of the slurry of conditioned SBM to pH 5, bran and corn were added, and the mixture incubated at 45 C for 8h. The pH of the incubated mixtures remained near 5 when checked after the 8h enzymolysis reaction.

Table V shows that conditioning one part of SBM with 8.3 parts of water and 0.015 parts of oxalic acid at pH 4.5 gave 75% destruction of phytate, after mixing with ground corn and bran and incubating in the usual way. Extending the SBM conditioning period from 2h to 4h and the enzymolysis period from 8h to 12h did not increase loss of Pp. Surprisingly, conditioning SBM at pH 8.5 with 8.3 parts of water and 0.0075 parts of oxalic acid added to the slurry gave only 51% loss (Table V) of Pp in the ternary mixture undergoing enzymolysis (pH 5, 45 C, 8h), compared to 65% destruction (Table IV) in the binary mixture of SBM and bran (enzymolysis at pH 5, 45 C, 6h) in which SBM had been conditioned in 4 parts of water at pH 8.5 containing 0.02 parts of sodium carbonate. Apparently, oxalic acid is a poor chelator of Ca^{++} or Mg^{++} at pH 8.5, while Na_2CO_3 at that pH causes those cations to precipitate as carbonate salts.

Conditioning a Mixture of SBM and Ground Corn Followed by

 Enzymolysis of the Ternary Mixture of Conditioned SBM,

Conditioned Corn and Bran

A 1:1 mixture of SBM and ground corn was conditioned at 55 °C and pH 4.5, 5.5, 6.5 with 4.2 parts of water and 0.0075 parts of oxalic acid based on the mixture of SBM and corn. The conditioned mixture was adjusted to pH 5, mixed with bran, and then allowed to incubate at 45 °C for 8h. The data in Table VI show that using a conditioning pH of 4.5 with oxalic acid, the loss of Pp, after enzymolysis of the ternary mixture was 75%. However, the data in Table V show that destruction of Pp was about 75% when SBM (12g) alone was conditioned at pH 4.5 in the presence of 0.18g of oxalic acid. Thus, different soaking conditions appeared to be needed for corn vs SBM.

The data in Table VII show that 80%±1% destruction of phytate was achieved after enzymolysis of the ternary mixture when the SBM and ground corn were preconditioned at pH 4.5 and 3.5, respectively. The conditioning medium for each ingredient contained 4.2 parts of water and 0.75% oxalic acid based on the ingredient, and the conditioning temperature was 55 °C. Eighty two percent destruction of Pp was achieved if the corn and SBM were conditioned sequentially in the same container (Table VIII). Corn was first conditioned in 8.3 parts of water with 0.015 parts of oxalic acid at pH 3.5 and 55 °C for 2h, then SBM was added, the pH adjusted with HCl to pH 4.5, and the mixture conditioned at 55 °C for an additional 2h. The solid-ingredients:water ratio in the final 2h of conditioning was 1:4.2.

In the experiments to determine preliminary conditions for releasing phytate from protein in SBM and corn, only one sample and one assay was used. But when the separate conditioning

method and the sequential conditioning method with the best conditions for destruction of phytate (over 70% loss of Pp) were found, some triplicate samples were run. The losses of Pp were indicated by the average values and the deviation, which included the conditioning, enzymolysis and assay steps.

In summary, Fig. 1 shows a flow chart of the best conditions of the separate conditioning method to destroy phytate in a mixture of the three principle ingredients of a growing chick feed, which gave $80\% \pm 1\%$ destruction of phytate. Fig. 2 shows the flow chart of the sequential conditioning method, which gave $82\% \pm 1\%$ destruction of phytate.

Assay of inorganic phosphorus before and after enzymolysis of the 4:4:1 mixture of corn:SBM:bran showed 78% of the phytate phosphorus was converted to orthophosphate. Apparently 22% of the Pp was present as intermediate phosphate esters of myo-inositol. Tanaka and Kasai (1981) found only inorganic P and myo-inositol and no intermediate phosphate esters when phytase attacked phytate in rice bran. They suggested phytase acted on the phytate in the periphery of the globoids (Yoshida et al, 1975). In this study, 78% of Pp was recovered as inorganic phosphorus, indicating a small amount of intermediate esters of myo-inositol formed in the enzymolysis reaction mixture.

After destruction of phytate, the 4:4:1 mixture of SBM:corn:bran contained 0.42% orthophosphate. The NRC (1977) requirement for growing chick feed is 0.4% Pi, which is normally added to the feed. The cost of the P-ingredient is about \$10-12 per ton, which is the third most costly supplement in the chick

feed (Allee, 1984). The cost in 1985 of the oxalic and hydrochloric acids needed to achieve 83% Pp destruction is \$5.8 per ton of feed ingredients. The processing cost were unknown.

Loss of Phytate Phosphorus during Ensiling of Whole Corn

Ensiling may be another way to destroy phytate in corn. Muirhead (1984) reported that phosphorus in high moisture corn (relative availability of phosphorus was 40%) stored 28 days in air-tight bags was two to three times more available than the phosphorus in dry corn (relative availability of phosphorus was 18%). The increase in bone strength of pigs fed the corn diets vs monosodium phosphate as standard was used to estimate the relative availability of phosphorus in corn, according to the reporter.

In the work reported here, 40lb of whole corn was divided into 4 equal parts, and each part was placed in a double walled polyethylene bags stored in a dark hardboard container. Phytate phosphorus and orthophosphate was followed in the grain with storage time (Table IX).

The data in Table IX show that Pp loss was low except after the 5th week, which showed 24% loss of Pp. The gain in orthophosphate accounted for only one-half the stoichiometric loss of Pp. Thus, some intermediate higher esters of myo-

inositol accumulated in the corn.

CONCLUSIONS

(1) A 4:4:1 mixture of soybean meal, ground corn and bran was made 82% free of Pp by using a two-step process. In step I,

corn and SBM were sequentially wet conditioned at pH 3.5 and 4.5, respectively, in the presence of 0.18% oxalic acid (based on water). In step II, the conditioned SBM and corn were mixed, brought to pH 5 using NaOH (2N), and bran was added to the slurry to provide phytase for enzymatic hydrolysis at 45 °C for 8h. The ratio of solid-ingredients:water used in the conditioning and the enzymolysis steps are 1:4.2 and 1:3.3, respectively. During the enzymic process, 78% of Pp was converted to orthophosphate in the dried reaction mixture.

(2) Ensiling whole corn for 5 weeks at 30% moisture gave 24% loss of Pp, only one-half of which appeared as orthophosphate.

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Table I The Composition^a of the Major Feed Ingredients.

Ingredients	Moisture %	Protein %	Ash %	Pp %	Phytic acid,%	Amount in Feed,%	Pp from Ingredient in Feed,%
					^b	^c	^d
Wheat bran	14.8	17.6	7.1	1.4	4.97	10	30
SBM	12.6	50.4	7.5	0.54	1.92	40	47
Ground Corn	12.8	10.6	1.7	0.26	0.92	40	23

a All percentages reported on a dry-weight basis, except moisture.

b Phytic acid calculated by the equation $(Pp, \text{ mg}) \times (3.546) = \text{phytic acid, mg}$ (Tangkongchitr et al., 1980a), then converted to percentage.

c The remaining 10% of feed is vitamin and mineral premixes.

d The total Pp in the mixture was 511mg per 100g mixture.

Table II Loss of Phytate Phosphorus (Pp) During Incubation Individually of Wet Bran, Soybean Meal (SBM) and Ground Corn, or During Incubation of a Mixture of Bran and SBM, or Bran and Corn.

Ingredients	Ratio of Ingredients/Water during Enzymolysis	Enzymolysis Conditions	Loss of Pp %
Bran	1/3.2	pH 5, 45 C, 6h	91±2 ^a
Soybean Meal (SBM)	1/3.2	pH 5, 45 C, 6h	10±2
Corn (ground)	1/3.2	pH 5, 45 C, 6h	21±2
SBM and Bran(4:1)	1/3.2	pH 5, 45 C, 6h	37±1
Corn and Bran(4:1)	1/3.2	pH 5, 45 C, 6h	51±4

a Pp loss calculated based on total Pp in the ingredient(s).
 Triplicate samples of ingredient(s) treated and then assayed for Pp.

Table III ^a Phytate Destroyed in a Binary Ingredient Mixture
 (4:1) of Conditioned SBM and Bran Incubated for 6h
 at 45 C and pH 5.

Conditioning pH	Ratio of SBM/Water	Ratio of SBM+Bran/Water during Enzymolysis	Loss of Pp in the Mixture of SBM and Bran (%)		
			Temp. of 45°C	55°C	65°C
2	1/2.5	1/2.0	<10	--	--
3	1/3.3	1/2.6	45 (43) ^b	--	--
4	1/3.8	1/3.0	58 (57)	--	--
5	1/3.9	1/3.1	49	--	--
6	1/4.0	1/3.2	45	--	--
7	1/4.0	1/3.2	53	56	58
8	1/4.0	1/3.1	56	58	60 (60)
9	1/3.9	1/3.1	39 (46)	--	40
10	1/3.5	1/2.8	35	--	--

a In the conditioning step the soaking time was 2h. In the enzymatic hydrolysis step, conditioned SBM (24g, "as is" m.b.) was adjusted to pH 5, mixed with bran (6g) and the mixture allowed to stand 6h at 45 C. The SBM and bran contained 0.54% and 1.4% Pp, respectively, and the loss of Pp was calculated from the sum of Pp in the mixture.

b The values in parentheses are the losses of Pp obtained using a 3h conditioning period instead of 2h.

Table IV ^a Phytate Destroyed in a Binary Ingredient Mixture (4:1) of SBM and Bran Incubated at a Solid-Ingredients/Water Ratio of 1/3.2 for 6h at 45 C and pH 5. The SBM Was Preconditioned in the Presence of Na CO_{2 3} with or without a Chelating Agent.

Conditioning		Enzymolysis		Loss of Pp in the Mixture of SBM and Bran, %	
EDTA	Oxalic Acid	EDTA	Oxalic Acid	Temp. of Conditioning	
pH	g/100g of SBM	g/100g of SBM+Bran	g/100g of SBM+Bran	55°C	65°C
7.5	--	--	--	55	57
8	--	--	--	66	63
8.3	--	--	--	69 (69) ^b	68
8.5	--	--	--	65 (71)	62
8.5	1.2	--	--	(73)	--
8.5	--	0.75	--	(69)	--
8.5	--	--	0.97	(86)	--
8.5	--	--	--	0.60	(74)
8.7	--	--	--	(61)	--
8.9	--	--	--	(68)	--
9.1	--	--	--	(67)	--

a In the conditioning step, SBM (24g, "as is" m.b.) was soaked in water (95-98ml) at various pH's for 2h at 55 C or 65 C. In the enzymolysis step, the conditioned SBM (24g) slurry was adjusted to pH 5 (after 30min standing) using 2-4ml of 5N HCl, mixed with bran (6g), and the mixture allowed to stand 6h at 45 C. EDTA or oxalic acid was sometimes added at the

Table IV (Continued)

conditioning and enzymolysis step.

b The values in parentheses are the percentages of loss of Pp for an 8h enzymolysis reaction.

Table V ^a Phytate Destroyed in a Ternary Ingredient Mixture (4:4:1) of SBM, Ground Corn and Bran Incubated at a Solid-Ingredients/Water Ratio of 1/3.7 for 8h at 45 °C and pH 5. The SBM Was Preconditioned in the Presence of Oxalic Acid at a SBM/Water Ratio of 1/8.3 and at Different pH's.

Conditioning of SBM		Loss of Pp in the
pH	Oxalic acid, g/100g of SBM	Mixture of SBM, Corn and Bran %
	^b	^c
4.5	0.75	65 (63)
4.5	1.5	75
5.5	0.75	70 (67)
5.5	1.5	73
6.5	0.75	63 (62)
6.5	1.5	69
8.5	0.75	51

a In the conditioning step, SBM (12g, "as is" m.b.) was soaked in water (100ml) at various pH's for 2h at 55 °C. In the enzymolysis step, the conditioned SBM slurry was adjusted to pH 5 (after 30min standing) using either 5N HCl or 2N NaOH, mixed with ground corn (12g) and bran (3g), and the mixture allowed to stand 8h at 45 °C.

b The quantity of oxalic acid was 0.09g or 0.18g for 12g of SBM.

c The values in parentheses are the percent of destruction of phytate after 4h in the conditioning step and 12h in the enzymolysis step.

Table VI ^a Phytate Destroyed in a Ternary Ingredient Mixture (4:4:1) of SBM, Ground Corn and Bran Incubated at a Solid-Ingredients/Water Ratio of 1/3.7 for 8h at 45 °C and pH 5. The mixture of SBM and Ground Corn was Conditioned Together in the Presence of 0.75% Oxalic Acid (Based on Solid Ingredients) at Different pH's Using a Solid-Ingredients/Water Ratio of 1/4.2.

Conditioning		Loss of Pp in the Mixture of SBM, Corn and Bran %
pH	Oxalic acid, g/100g of SBM+Corn(1:1)	
4.5	0.75	75
5.5	0.75	64
6.5	0.75	63

^a In the conditioning step, SBM (12g, "as is" m.b.), corn (12g) and oxalic acid (0.18g) were combined in water (100ml) and the mixture was soaked for 2h at 55 °C. In the enzymolysis step, the conditioned mixture of SBM and corn was adjusted to pH 5 (after 30min standing), mixed with bran (3g), and the mixture allowed to stand 8h at 45 °C.

Table VII Phytate Destroyed in a Ternary Ingredient Mixture of SBM, Ground Corn and Bran Incubated at a Solid-Ingredients/Water Ratio of 1/3.7 for 8h at 45 °C and pH 5. Ground Corn and SBM Were Conditioned in Separate Containers Prior to Enzymolysis.

Corn Conditioning ^a		SBM Conditioning ^a		Loss of Pp in the Ternary Mixture of SBM, Corn and Bran, %
pH	Time, h	pH	Time, h	
3.0	2	4.5	2	75±1 ^b
3.5	1	4.5	2	80±1 ^b
3.5	3	4.5	2	75
4.0	1-3	4.5	2	72-73
4.0	2	4.5	2	77

a Corn (12g, "as is" m.b.) or SBM (12g) was mixed with water (50ml) containing 0.09g of oxalic acid, and pH was adjusted using 5N HCl (0.2ml-1.2ml). After conditioning for 2h at 55 °C, the conditioned slurries were combined, and the mixture adjusted to pH 5 using 2N NaOH (1.2-2.4ml). Bran (3g) was added, and the mixture then allowed to undergo enzymolysis 8h at 45 °C.

b Triplicate samples conditioned, subjected to enzymolysis, then assayed for Pp.

Table VIII Phytate Destroyed in a Ternary Ingredient Mixture of SBM, Ground Corn and Bran Incubated at a Solid-Ingredients/Water Ratio of 1/3.7 for 8h at 45°C and pH 5. Ground Corn and SBM Were Sequentially Conditioned Prior to Enzymolysis.

Corn Conditioning ^a		Mixture Conditioning ^a		Loss of Pp in the Ternary Mixture of SBM, Corn and Bran, %
pH	Temp. °C	pH	Temp. °C	
3.0	55	4.5	55	79±2 ^b
3.0	65	4.5	55	76
3.5	55	4.5	55	82±1 ^b
3.5	65	4.5	55	77
4.0	55	4.5	55	75

a Corn (12g, "as is" m.b.) was conditioned for 2h in water (100ml) containing 0.18g of oxalic acid. Then, SBM (12g) was added, the pH adjusted using 5N HCl (0.4-0.6ml), and the mixture of SBM and corn was conditioned another 2h at 55°C (solid-ingredients/water ratio of 1/4.2). After the conditioning step, the conditioned slurry was adjusted to pH 5 using 2N NaOH (0.4ml). Bran (3g) was then added, and the mixture allowed to stand 8h at 45°C.

b Triplicate samples conditioned, subjected to enzymolysis, and then assayed for Pp.

Table IX ^a Loss of Phytate and Gain of Orthophosphate Ensiled
Whole Corn at 30% Moisture.

Week	Pp(%)			Phytate Loss(%)			Pi (%)		
	T	C	B	T	C	B	T	C	B
1	0.22	0.23	--	12	8	--	0.05	0.06	--
2	0.22	0.23	0.22	13	8	13	0.06	0.06	0.08
3	0.21	0.22	0.22	16	12	10	0.08	0.08	0.08
5	0.19	0.19	0.19	23	22	24	0.10	0.10	0.10

a T,C,B are the samples for corn removed from top, center,
bottom of a bag. The ensiling temperature was 21 °C.

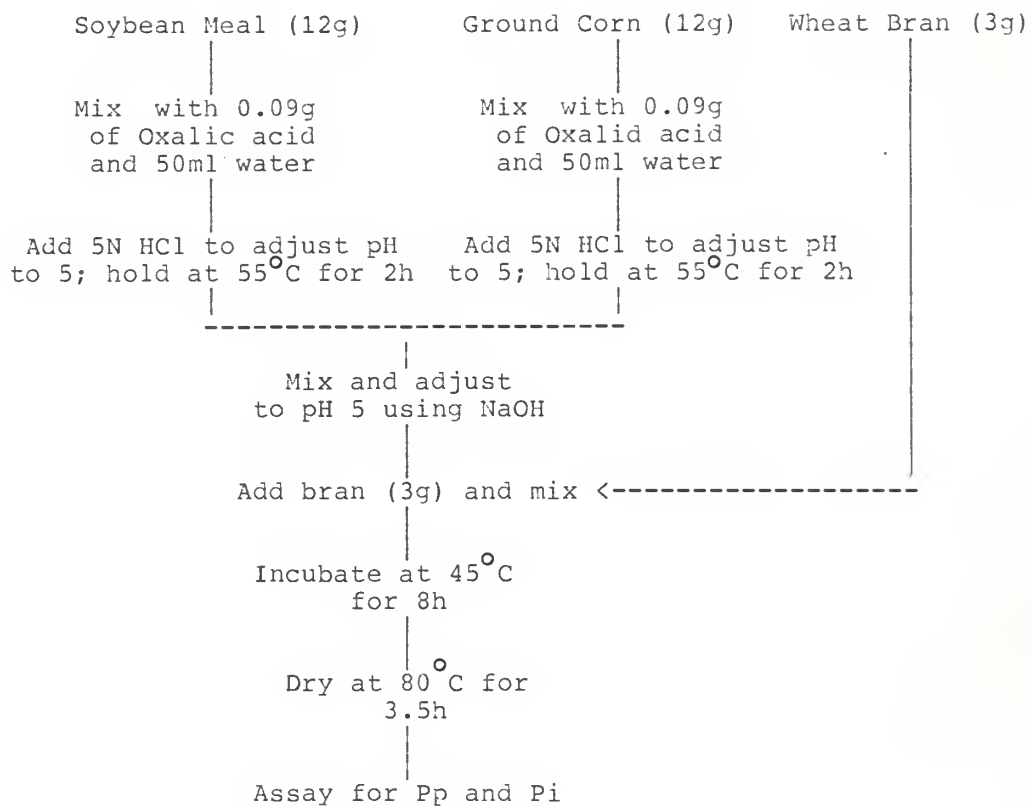


Fig. 1 Scheme to Destroy Phytate in a Ternary Ingredient Mixture of Soybean Meal, Ground Corn and Wheat Bran Using the Separate Conditioning of SBM and Corn

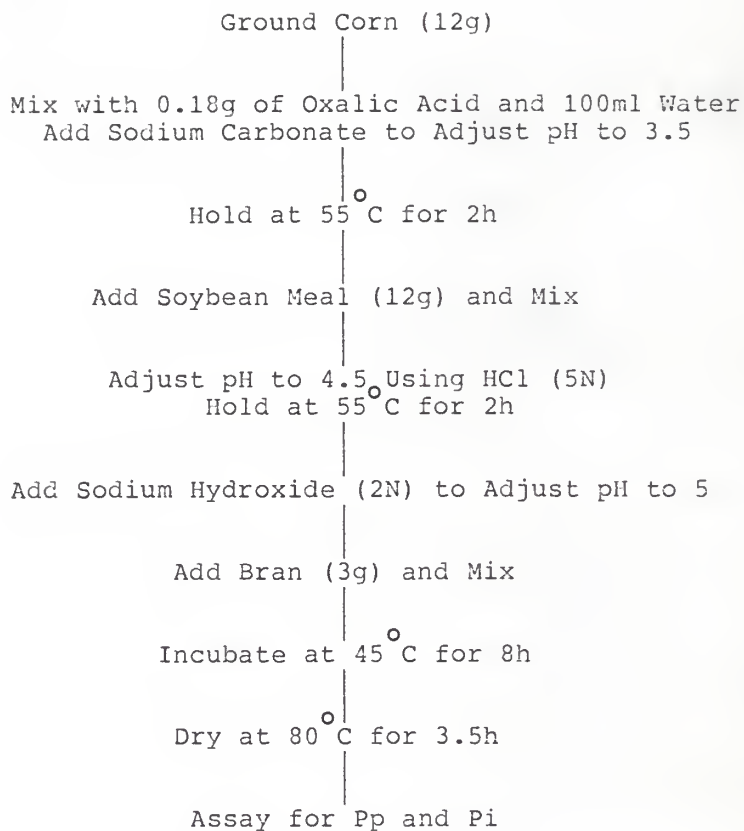


Fig. 2 Scheme to Destroy Phytate in a Ternary Ingredient Mixture of Soybean Meal, Ground Corn and Wheat Bran Using the Sequential Conditioning of SBM and Corn

APPENDIX

It was noticed that upon adjusting pH of mixtures of SBM and bran, the pH-values changed during the first 30 min after adjustment, but then stabilized after 2-6h. The pH values during pH adjustment at the conditioning and the enzymolysis are shown in Table X. The pH values at the 30 min. point were reported as the pH used during the two steps.

Table X Change of pH During Conditioning and Enzymolysis

pH	Conditioning			Enzymolysis		
	pH at Beginning	pH at 30 min Later	pH after 2h	pH at Beginning	pH at 30 min Later	pH after 6h
8	8.9	8	7.8	4.5	4.9	5.1
8.3	9.9	8.3	7.8	4.5	5	5.05
8.3	9.9	8.3	7.9	4.3	5	5.1
8.5	10.1	8.5	8.3	4.3	4.9	5
8.5	10.1	8.5	8.4	4.4	4.9	5.1
8.7	10.3	8.7	8.35	4.4	5.05	5.1
8.9	10.4	8.9	8.55	4.4	4.85	5

DESTRUCTION OF PHYTATE IN A WET MIXTURE
OF SOYBEAN MEAL, GROUND CORN AND BRAN

by

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AN ABSTRACT OF A MASTER'S THESIS

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Phytate in a 4:4:1 mixture of soybean meal (SBM), ground corn and wheat bran was hydrolyzed by the native enzyme in bran to an extent of 83% by a two-step process. In the conditioning step, ground corn (1 part, "as is" m.b.) was conditioned at 55 °C and pH 3.5 in 8.3 parts of water containing 0.18% oxalic acid (based on water) and 0.036g of Na₂CO₃. After 2h, the slurry was adjusted pH to 4.5 using 0.11% of 5N HCl (based on water), SBM (1 part) was then added. The wet mixture of ground corn and SBM was conditioned for another 2h at 55 °C and pH 4.5. In the enzymolysis step, the wet-acid conditioned SBM and corn were mixed together, adjusted to pH 5, and bran (0.25 parts) added. The ternary ingredient mixture was held 8h at 45 °C and pH 5 to enzymically hydrolyze phytate. The orthophosphate released into the reaction mixture accounted for 78% of phytate phosphorus released. On a dry weight basis the 4:4:1 mixture of SBM, ground corn and bran contained 0.42% inorganic phosphorus after the enzymic reaction. The 1985 cost of oxalic acid and HCl needed to treat 2000lb of ternary mixture was \$5.8. Ensiling whole corn at 21 °C and 30% moisture gave little destruction of phytate until 5 weeks, where 24% Pp was lost.